

WHAT IS CLAIMED:

1. A transgenic non-human animal whose somatic cells and germ cells are homozygous for an altered MC-3R gene which encodes a non-functional MC-3R protein.

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2. A cell line derived from a transgenic animal of claim 1.

3. A transgenic mouse whose somatic cells and germ cells are homozygous for an altered MC-3R gene which encodes a non-functional MC-3R protein.

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4. A transgenic mouse of claim 3 wherein said mouse exhibits a disorder selected from the group consisting of an obesity syndrome, diabetes, male and female sexual dysfunction, pain, memory, neuronal regeneration and neuropathy, growth disorders relating to reduced GH, IGF1 function, and other states resulting from GH deficiency.

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5. The transgenic mouse of claim 4 wherein said mouse exhibits an obesity syndrome.

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6. A cell line derived from a transgenic mouse of claim 3.

7. A cell line derived from a transgenic mouse of claim 4

8. A cell line derived from a transgenic mouse of claim 5.

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9. The mouse of claim 6, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene to its offspring.

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10. The mouse of claim 7, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene to its offspring.

11. The mouse of claim 8, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene to its offspring.

12. A transgenic mouse whose somatic cells are heterozygous for a functional murine gene coding for a MC-3R protein and an altered MC-3R gene.

5 13. A transgenic mouse of claim 12 wherein said mouse exhibits a disorder selected from the group consisting of an obesity syndrome, diabetes, male and female sexual dysfunction, pain, memory, neuronal regeneration and neuropathy, growth disorders relating to reduced GH, IGF1 function, and other states resulting from GH deficiency.

10 14. The transgenic mouse of claim 13 wherein said mouse exhibits an obesity syndrome.

15 15. A cell line derived from a transgenic animal according to Claim 12.

16. A cell line derived from a transgenic animal according to Claim 13.

17. A cell line derived from a transgenic animal according to Claim 14.

20 18. The mouse of claim 15, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene to its offspring.

19. The mouse of claim 16, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene to its offspring.

25 20. The mouse of claim 17, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene to its offspring.

30 21. A transgenic mouse whose somatic cells are hemizygous for an altered MC-3R gene.

22. A transgenic mouse of claim 21 wherein said mouse exhibits a disorder selected from the group consisting of an obesity syndrome, diabetes, male and female sexual dysfunction, pain, memory, neuronal regeneration and neuropathy, growth

disorders relating to reduced GH, IGF1 function, and other states resulting from GH deficiency.

5 23. The transgenic mouse of claim 22 wherein said mouse exhibits an obesity syndrome.

24. A cell line derived from a transgenic animal according to Claim 21.

10 25. A cell line derived from a transgenic animal according to Claim 22.

26. A cell line derived from a transgenic animal according to Claim 23.

15 27. The mouse of claim 24, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene to its offspring.

28. The mouse of claim 25, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene to its offspring.

20 29. The mouse of claim 26, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene to its offspring.

25 30. A transgenic mouse whose somatic cells and germ cells lack a functional gene coding for a murine MC-3R protein and which contain and express a transgene comprising a gene for a non-native MC-3R protein, wherein said mouse is viable.

31. The transgenic mouse of claim 30 wherein said non-native MC-3R transgene encodes wild-type human MC-3R.

30 32. The transgenic mouse of claim 31 wherein said non-native MC-3R transgene encodes a mutated form of human MC-3R.

33. A method of producing a mouse having somatic and germ cells that lack a murine gene coding for MC-3R, which comprises:

- (a) providing a gene encoding an altered form of MC-3R designed to target a MC-3R allele of mouse embryonic stem cells;
- (b) introducing the altered gene into mouse embryonic stem cells;
- (c) selecting embryonic stem cells which contain the altered gene;
- 5 (d) introducing the embryonic stem cells containing the altered gene into mouse blastocysts;
- (e) transplanting the injected blastocysts into a pseudopregnant mouse, and
- (f) allowing the embryo to develop to term; to produce a chimeric founder transgenic mouse.

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34. The method of Claim 33 wherein the introduction of step (d) is by microinjection.

35. The method of Claim 33 which further comprises the steps:

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(g) breeding chimeric transgenic mice with wild-type mice to obtain F1 mice heterozygous for said altered MC-3R gene.

36. A transgenic non-human animal whose somatic cells and germ cells are homozygous for an altered MC-3R gene which encodes a non-functional MC-3R protein and homozygous for an altered MC-4R gene which encodes a non-functional MC-4R protein.

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37. A cell line derived from a transgenic animal of claim 36.

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38. A transgenic mouse whose somatic cells and germ cells are homozygous for an altered MC-3R gene which encodes a non-functional MC-3R protein and homozygous for an altered MC-4R gene which encodes a non-functional MC-4R protein.

39. A cell line derived from a transgenic mouse of claim 38.

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40. The mouse of claim 39, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene and MC-4R gene to its offspring.

41. A transgenic mouse whose somatic cells are heterozygous or homozygous for an altered MC-3R gene and an altered MC-4R gene, wherein an altered gene expresses a non-functional protein.

5 42. A cell line derived from a transgenic animal according to Claim 41.

43. The mouse of claim 41, wherein the mouse is fertile and capable of transmitting the altered MC-3R gene and/or MC-4R gene to its offspring.

10 44. A transgenic mouse whose somatic cells and germ cells lack a functional gene coding for a murine MC-3R protein and MC-4R protein and which contain and express a transgene comprising a gene for a non-native MC-3R protein and a non-native MC-4R protein, wherein said mouse is viable.

15 45. The transgenic mouse of claim 44 wherein said non-native MC-3R transgene encodes wild-type human MC-3R and wild-type human MC-4R.

46. The transgenic mouse of claim 44 wherein said non-native MC-3R or MC-4R transgene encodes a mutated form of the protein.

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47. A method for determining whether a substance is capable of binding to MC-3R comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of MC-3R in the cells;

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(b) exposing the test cells to the substance;

(c) measuring the amount of binding of the substance to MC-3R;

(d) comparing the amount of binding of the substance to MC-3R in the test cells with the amount of binding of the substance to control cells that have not been transfected with MC-3R, wherein a substance which binds to MC-3R is identified as a substance which potentially regulates body weight.

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48. A method for determining whether a substance is capable of activating MC-3R and regulating body weight, comprising:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of MC-3R in the cells;
- (b) exposing the test cells to the substance;
- (c) measuring the amount of accumulated intracellular cAMP;
- 5 (d) comparing the amount of cAMP in the test cells in response to the substance with the amount of cAMP in test cells that have not been exposed to the substance, wherein a substance which binds to MC-3R is identified as a substance which potentially regulates body weight.

10 49. A method of identifying a substance which modulates MC-3R receptor activity and regulate body weight, comprising:

- (a) combining a test substance in the presence and absence of a MC-3R receptor protein wherein said MC-3R receptor protein comprises the amino acid sequence as set forth in SEQ ID NO:4; and,
- 15 (b) measuring and comparing the effect of the test substance in the presence and absence of the MC-3R receptor protein.

50. A method for determining whether a substance is a potential agonist or antagonist of MC-3R and regulates body weight, comprising:

- 20 (a) transfecting or transforming cells with an expression vector that directs expression of MC-3R in the cells, resulting in test cells;
- (b) allowing the test cells to grow for a time sufficient to allow MC-3R to be expressed;
- (c) exposing the cells to a labeled ligand of MC-3R in the presence and in
- 25 the absence of the substance;
- (d) measuring the binding of the labeled ligand to MC-3R; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of MC-3R.

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51. A method for determining whether a substance is capable of binding to MC-3R and regulating body weight, comprising:

- (a) transfecting or transforming cells with an expression vector that directs the expression of MC-3R in the cells, resulting in test cells;

- (b) exposing the test cells to the substance;
 - (c) measuring the amount of binding of the substance to MC-3R;
 - (d) comparing the amount of binding of the substance to MC-3R in the test cells with the amount of binding of the substance to control cells that have not
- 5 been transfected with MC-3R;

wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to MC-3R.

52. A method for determining whether a substance is capable of binding to MC-3R and regulating body weight, comprising:

- (a) transfecting or transforming cells with an expression vector that directs the expression of MC-3R in the cells, resulting in test cells;
 - (b) preparing membranes containing MC-3R from the test cells and exposing the membranes to a ligand of MC-3R under conditions such that the ligand
- 15 binds to the MC-3R in the membranes;
- (c) subsequently or concurrently to step (b), exposing the membranes from the test cells to a substance;
 - (d) measuring the amount of binding of the ligand to the MC-3R in the membranes in the presence and the absence of the substance;
- 20 (e) comparing the amount of binding of the ligand to MC-3R in the membranes in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to MC-3R in the membranes in the presence of the substance indicates that the substance is capable of binding to MC-3R.

25 53. A method for determining whether a substance is capable of binding to MC-3R and regulating body weight, comprising:

- (a) transfecting or transforming cells with an expression vector that directs the expression of MC-3R in the cells, resulting in test cells;
 - (b) preparing membranes containing MC-3R from the test cells and
- 30 exposing the membranes from the test cells to the substance;
- (c) measuring the amount of binding of the substance to the MC-3R in the membranes from the test cells;
 - (d) comparing the amount of binding of the substance to MC-3R in the membranes from the test cells with the amount of binding of the substance to

membranes from control cells that have not been transfected with MC-3R, where if the amount of binding of the substance to MC-3R in the membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to MC-3R.

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54. A method of identifying agonists of MC-3R which regulate body weight comprising:

(a) transfecting or transforming cells with a first expression vector which directs expression of MC-3R and a second expression vector which directs the expression of a promiscuous G-protein, resulting in test cells;

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(b) exposing the test cells to a substance that is a suspected agonist of MC-3R;

(c) measuring the level of inositol phosphates in the cells;

where an increase in the level of inositol phosphates in the cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist indicates that the substance is an agonist of MC-3R.

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55. A method of identifying antagonists of MC-3R which regulate body weight comprising:

(a) transfecting or transforming cells with a first expression vector which directs expression of MC-3R and a second expression vector which directs the expression of a promiscuous G-protein, resulting in test cells;

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(b) exposing the test cells to a substance that is an agonist of MC-3R;

(c) subsequently or concurrently to step (b), exposing the test cells to a substance that is a suspected antagonist of MC-3R;

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(d) measuring the level of inositol phosphates in the cells;

where a decrease in the level of inositol phosphates in the cells in the presence of the suspected antagonist as compared to the level of inositol phosphates in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of MC-3R.

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56. A method of identifying antagonists of MC-3R as recited in claim 55 wherein the first and second expression vectors of step (a) are replaced with a single

expression vector which expresses a chimeric MC-3R protein fused at its C-terminus to a promiscuous G-protein.

57. A method of selecting for a compound which shows *in vivo* efficacy for modulation of MC-3R and regulation of body weight which comprises administering a compound selected by the method of claims 47-56 to a non-human animal to measure the effect administering the compound has on the regulation of body weight within the non-human animal.

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